

IN THE CLAIMS

1. (Currently Amended) A method for regeneration of cotton via somatic embryogenesis with substantially synchronized development of embryos after short duration inositol starvation, said process comprising the steps of:

- (i) cutting from the germinated cotton seedling the explant, selected from a group consisting of cotyledon, hypocotyl, and mesocotyl and or mixtures thereof;
- (ii) culturing the explant for the purpose of callus induction in a first solid medium, in a culture medium containing glucose as the carbon source supplemented with Gamborg B5 vitamins 2,4-D, and BA and inositol, at a temperature between 23 to 33°C in light intensity of at least 90 $\mu\text{mol/m}^2/\text{s}$ under a 16 hour ~~photo~~period photoperiod for a period of 3-5 weeks, to enable dedifferentiated callus to form from the explant;
- (iii) transferring the callus from the first solid callus induction medium to a liquid medium comprising a basal medium containing glucose as the carbon source and supplemented with Gamborg B5 vitamins and culturing the suspension generated thereof at a temperature from 23 to 33°C in a reduced light intensity of 20-40 $\mu\text{mol/m}^2/\text{s}$, under a 16 hour photoperiod for a period of time sufficient to form embryogenic clumps;
- (iv) screening the cell suspension through metal sieves of different pore sizes to select embryogenic cells and/or clumps and subculturing the embryogenic callus containing somatic embryos to said basal medium;
- (v) subjecting the embryogenic mass / clumps to inositol deprivation, consequent upon subculturing it to said basal medium devoid of inositol ~~for a sufficient period of time~~ and then returning the culture to inositol containing medium to enable the somatic embryos to synchronize developmentally;
- (vi) transferring bipolar somatic embryos to an embryo germination medium on a support and growing the embryos in embryo germination medium up to the plantlet stage developed sufficiently for transfer to soil;
- (vii) further transferring the plantlets to a potting mix for acclimatization and then to field.

2. (Currently Amended) ~~A~~ The method as claimed in claim 1, wherein the explants are derived from cotton or any other plant seedlings.

3. (Currently Amended) The method as recited in claim 1, wherein the explant is derived from cotton cv Coker 312 and the seedlings are developed by:

(i) sterilizing cotton seed in a sterilization solution of 0.1% HgCl_2 for 5-10 min., ~~preferably 7 min.,~~

(ii) rinsing the seed in sterile water 4-6 times,

(iii) scorching the seed in flame of a spirit burner for 5-10 seconds,

(iv) inoculating the seed on seed germination medium,

(v) growing the seed in the seed germination medium in light or dark at a temperature of ~~23 degree to 33 degree C~~ 23° to 33°C for a period of 6-12 days, ~~preferably 9-10 days, and~~

(vi) excising the explant from the seedling.

4. (Currently Amended) ~~A~~ The method as claimed in claim 3, wherein seed germination medium is a liquid medium comprising salts of Murashige and Skoog and Gamborg B5 vitamins at half of its concentration.

5. (Currently Amended) ~~A~~ The method as claimed in claim 3, wherein carbon source in seed germination medium is selected from a group consisting of sucrose and glucose at a range of 1 to 3% wt./ vol.

6. (Currently Amended) ~~A~~ The method as claimed in claim 1, wherein said first solid callus induction medium comprises following components of Murashige and Skoog medium:

Component	Conc. (mg/L)
a. Salts of Murashige and Skoog (1962) medium: -	
NH ₄ NO ₃	1650
KNO ₃	1900
CaCl ₂ .2H ₂ O	440
MgSO ₄ .7H ₂ O	370
KH ₂ PO ₄	170
KI	183 <u>0.83</u>
H ₃ BO ₃	6.2
MnSO ₄ H ₂ O	22.3
ZnSO ₄ .7H ₂ O	8.6
Na ₂ MoO ₄ .2H ₂ O	0.25
CuSO ₄ .5H ₂ O	0.025
CoCl ₂ .6H ₂ O	0.025
Na ₂ .EDTA	37.3
FeSO ₄ .7H ₂ O	27.8
b. Organics	
Myo-inositol	100

7.(Currently Amended) ~~A~~ The method as claimed in claim 1, wherein Gamborg B5 vitamins, wherever included comprise:

Component	Conc. (mg/L)
Nicotinic Acid	1.0
Pyridoxine HCl	1.0
Thiamine HCl	10

8. (Currently Amended) ~~A~~ The method as claimed in claim 1, wherein 2,4-D as exogenously supplied auxin in first solid callus induction medium is selected from a range of 0.44 to 4.4 μM [[;]] preferably ~~1.76 to 2.64 μM .~~

9. (Currently Amended) ~~A~~ The method as claimed in claim 1, wherein BA as exogenously supplied cytokinin in first solid callus induction medium is selected from a range of 0.22 μM to 2.2 μM [[;]] preferably ~~0.66 μM to 1.00 μM .~~

10.(Currently Amended) ~~A~~ The method as claimed in claim 1, wherein gelling agent in said first solid callus induction medium is selected from a group consisting of agar in the range of 0.6-0.8% wt./vol., preferably 0.7% and phytagel in the range of 0.15-0.29% wt./vol., preferably 0.22% wt./vol..

11.(Currently Amended) ~~A~~ The method as claimed in claim 1, wherein said first solid callus induction medium contains glucose as the primary carbon source.

12. (Currently Amended) ~~A~~ The method as claimed in claim 1, wherein said explants are cultured on said callus induction medium at a temperature between 23 to 33°C, preferably between 27 to 29°C in light intensity of at least 90 $\mu\text{mol}/\text{m}^2/\text{s}$ under a 16-h hour photoperiod for period of not more than of 3-5 weeks, to enable dedifferentiated callus to form from any of the ~~said~~ explant.

13.(Currently Amended) ~~A~~ The method as claimed in claim 1, essentially including the step of transferring callus from the said first solid callus induction medium to a liquid medium in Ehrlenmeyer flasks at a packing density of 600 to 1000 mg of callus/50 ml of media preferably, ~~800 mg/50 ml~~

and shaking the culture in this and all subsequent steps until somatic embryos are taken out for germination on a gyratory shaker at 110-130 rpm.

14. (Currently Amended) ~~A~~ The method as claimed in claim 1, wherein said embryogenesis induction medium is a basal liquid medium comprising ~~M&S~~ Murashige and Skoog salts, Gamborg B5 vitamins, inositol and glucose as the carbon source.

15. (Currently Amended) ~~A~~ The method as claimed in claim 1, wherein plant cell suspension embryogenic mass and somatic embryos generated thereof in liquid medium are incubated at a temperature from 23 to 33°C, ~~preferably 27-29°C~~ in light intensity of 20-40 $\mu\text{mol}/\text{m}^2/\text{s}$, ~~typically 27-33 $\mu\text{mol}/\text{m}^2/\text{s}$~~ under a 16 h hour photoperiod.

16.(Currently Amended) ~~A~~ The method as claimed in claim 1, wherein said embryogenic mass/clumps are subjected to inositol deprivation for a period of 8 to 12 days, ~~preferably, 10 days~~ in inositol deprivation medium comprising MS basal salts, Gamborg B5 vitamins, glucose as carbon source but no inositol, leading to developmental synchronization of somatic embryos.

17. (Currently Amended) ~~A~~ The method as claimed in claim 1, wherein said first solid callus induction medium has a pH in the range of 5.4-6.2 and the entire liquid media in said process has a pH in the range of 5.2 - 5.8, being sterile as a result of autoclaving at 121°C, 16 psi for 16 minutes.

18. (Currently Amended) ~~A~~ The method as claimed in claim 1, wherein potting mix comprises of garden soil: sand: Peat moss: vermiculite typically in 2:1:1:1 ratio.

19.(Currently Amended) ~~A~~ The method as claimed in claim 1, wherein developmental synchrony of somatic embryogenesis is utilized for multiplication of elite cotton cultivar or development of transgenic cotton cultivar.

20. (Currently Amended) ~~A~~ The method as claimed in claim 1, wherein the inositol depletion is

applied to plant species other than cotton for enhancing embryogenesis in tissue culture.

21. (Currently Amended) ~~A~~ The method as claimed in claim 1, wherein said culture medium and basal medium comprise of Murashige and Skoog medium.

22. (Currently Amended) ~~A~~ The method as claimed in claim 1, wherein said period of time sufficient to from embryonic clumps comprises 12-32 days.

23.(Currently Amended) ~~A~~ The method as claimed in claim 1, wherein said subculturing the embryogenic callus containing somatic embryos to said basal medium is carried out at intervals of ~~8-12~~ 8-12 days.

24.(Currently Amended) ~~A~~ The method as claimed in claim 1, wherein said embryogenic mass/clumps are subjected to inositol deprivation for a period of 8 to 12 days, ~~preferably, 10 days~~.

25. (Currently Amended) ~~A~~ The method as claimed in claim 1, wherein said support for said embryo germination medium comprises vermiculite.

26. (New) The method according to part (v) of claim 3, wherein the seed is grown for 9-10 days.

27. (New) The method according to claim 15, wherein the plant cell suspension embryogenic mass and somatic embryos are incubated at a temperature from 27-29°C.

28. (New) The method according to claim 8, wherein the range is 1.76 to 2.64μM.

29. (New) The method according to claim 9, wherein the range is 0.66μM to 1.00μM.

30. (New) The method according to claim 12, wherein the explants are cultured on said callus induction medium at a temperature between 23 to 33°C.

31. (New) The method according to claim 15, wherein the temperature is from 27-29°C.

32. (New) The method according to claim 15, wherein the light intensity is 27-33 $\mu\text{mol}/\text{m}^2/\text{s}$.